Towards localization of engineered silver nanoparticles in Pseudokirchneriella subcapitata

Jensen, Louise Helene Søgaard; Sørensen, Sara Nørgaard; Hartmann, Nanna Isabella Bloch; Baun, Anders; Fleck, R.A.

Publication date:
2014

DTU Cen
Center for Electron Nanoscopy

Towards localization of engineered silver nanoparticles in *Pseudokirchneriella subcapitata*

Jensen LS[1], Sørensen SN[2], Hartmann NB[2], Baun A[2], Fleck RA[3]

1 Center for Electron Nanoscopy, Technical University of Denmark, Kgs. Lyngby, Denmark
2 Department of Environmental Engineering, Technical University of Denmark Kgs. Lyngby, Denmark
3 Centre For Ultra Structural Imaging, King's College London, London, United Kingdom

Silver nanoparticles have increased cytotoxic properties compared to larger particles. Reflecting these properties, engineered silver nanoparticles are now added to an increasing number of consumer products often labelled as anti-bacterial. These particles are presently considered the fastest growing nanotechnology application. Accordingly, silver nanoparticles are now postulated to be released into the sewerage systems and wider environment in increasing quantities. Here they could potentially interfere with aquatic life and this ongoing project aims to localize possible particles taken up by the freshwater algae *P subcapitata.*

Outline

Silver nanoparticles are postulated to be released into the sewerage systems and wider environment in increasing quantities because of an increase in the number of consumer products, often labelled as antibacterial, which contain engineered silver nanoparticles. These particles are presently considered the fastest growing nanotechnology application. Silver nanoparticles have increased cytotoxic properties compared to larger silver particles and there are concerns that they could inhibit the bacteria which are involved in the breakdown and processing of biological waste in wastewater treatment facilities and be harmful to aquatic organisms. Whether the enhanced toxicity of silver nanoparticles is due to an increased release of silver ions or it is related to additional mechanisms for toxicity is still a matter of scientific debate since there are studies supporting both theories. Furthermore, nanoparticles are highly heterogeneous in suspension and over time undergo processes such as aggregation, sedimentation, dissolution and changes in surface chemistry [1] – thus altering the dose and posing problems in standard experimental ecotoxicology model systems. Recently a modified short-term model has been suggested, which could potentially increase the accuracy of algal
growth inhibition tests with silver nanoparticles [2]. However, toxic mechanisms remain to be further elucidated and the uptake mechanism of the nanoparticles in aquatic organisms on an ultrastructural level play an important part of this. Selenastrum capricornutum Printz (1913) CCAP 278/4 (Pseudokirchneriella subcapitata (Korschikov) Hindák 1990) is a microalgae which is routinely applied in eco toxicity tests and has been used as a model organism in this study.

Aim

The aim of this study is to study whether silver nanoparticles are taken up by *P subcapitata* and to find the best method to locate possible nanoparticles inside the algal cells. For the location inside the cells, Focused Ion Beam Scanning Electron Microscopy (FIBSEM) and Serial Block-face Scanning Electron Microscopy (SBBEM) will be used and compared. However, this approach require a preparation protocol that confers high contrast to the samples. Hence, the first step has been to compare different protocols.

Setup

P subcapitata (10^4 cells / mL) were exposed to Ag nanoparticles (30 nm with citrate coating, 30 µg / L) for 72 hours at 20 °C and light intensity 100 ± 20 µmol*m^{-2}*s^{-1}.
A series of preparations were done (see Table 1) to estimate the contrast conferred by different protocols in order to create appropriate contrast for block-face imaging. Sections of similar thickness were imaged at similar conditions and normalized against the background. Finally the images were rated according to the contrast.
The algae were pelleted and fixed in 2% glutaraldehyde and 2% paraformaldehyde in 0,1 M Cacodylate buffer for 1 hour. They were then exposed to the conditions shown in Table 1. Finally they were dehydrated in a graded series of ethanol and infiltrated with Spurr's resin or Durcupan (hard recipes) and cut to ~70 nm sections on an ultramicrotome. They were imaged at 200 kV in a FEI Tecnai T20 G2.
Samples for FIBSEM were sectioned to an angle of 38 ° to fit the angle in the FIBSEM and subjected to Slice n' view in an FEI Helios Nanolab FIBSEM in immersion mode.

Table 1

Protocol number	Post fixation	Mordant	Double staining	En block staining
1	2% OsO_4 in 0.1 M Caco (1 h)	1% TA in milliQ water (1 h)	None	2% w/V UAc
2	1% OsO_4 + 1,5% KFeCN in 0.1 M Caco (1 h)	1% TA in milliQ water (1 h)	None	2% w/V UAc
3	1% OsO_4 + 1,5% KFeCN in 0.1 M Caco (1 h)	1% TA in milliQ water (1 h)	2% OsO_4 in milliQ water (30')	2% w/V UAc
4	1% OsO_4 + 1,5% KFeCN in 0.1 M Caco (1 h)	0.5 % TCH in ddH_2O (20')	2% OsO_4 in milliQ water (30')	2% w/V UAc
5	1% OsO_4 + 1,5% KFeCN in 0.1 M Caco (1 h)	1% TCH in ddH_2O (20')	2% OsO_4 in milliQ water (30')	2% w/V UAc

Figure 1

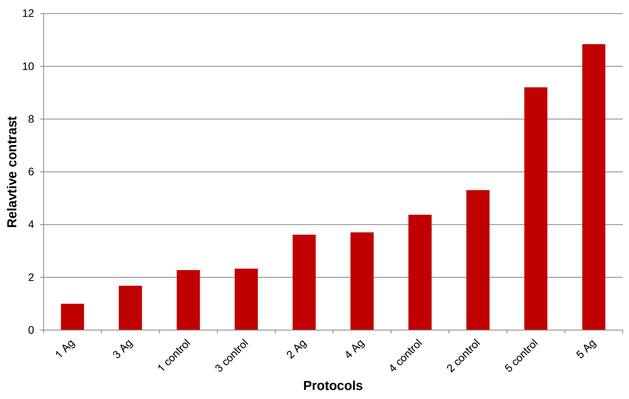

Figure 2

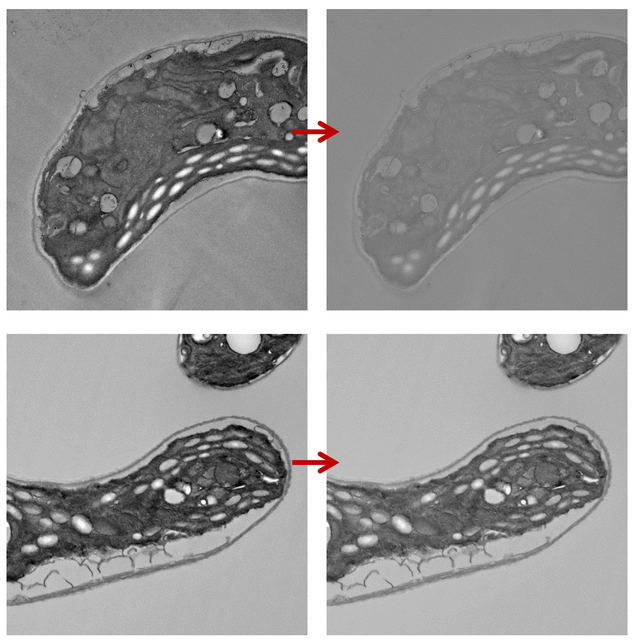

Table 1
Protocols used for different contrast. Caco: cacodylate buffer; TA: Tannic Acid; Uac: Uranyl acetate; TCH: ThioCarboHydrazide.

Figure 1
Contrast of different protocols. Contrast values are relative to the lowest contrast for comparison.

Figure 2
Example of images before and after being normalized against the background. Embedded in Dr. Spurr's resin. Examples are protocol 2 (top) and protocol 5 (bottom). HFW = 20.5 µm.

Figure 3

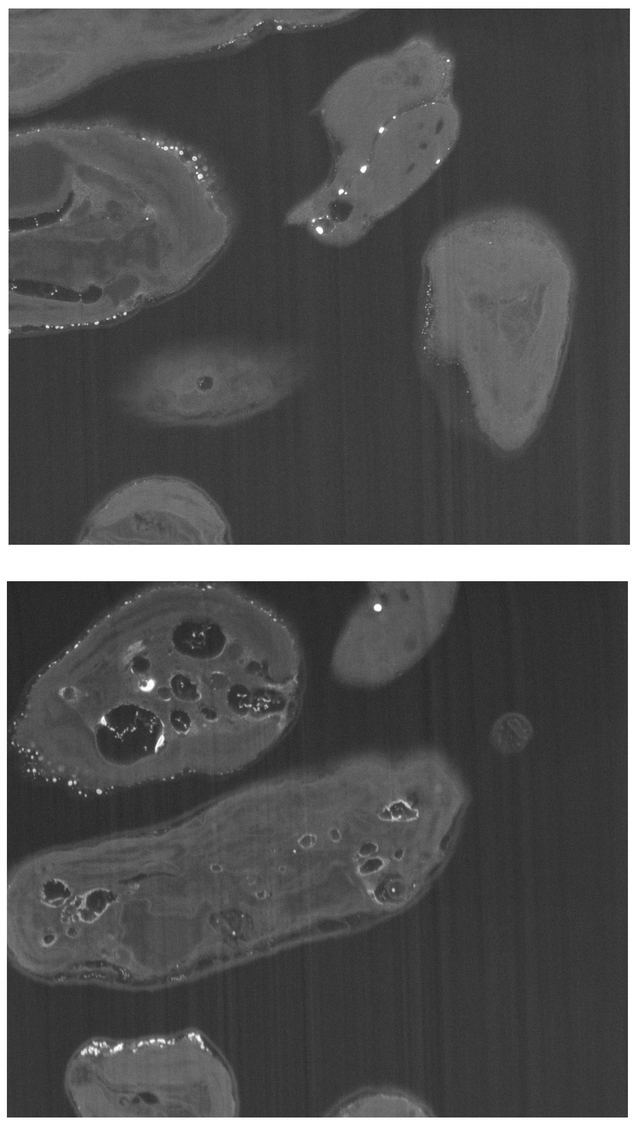

Figure 4

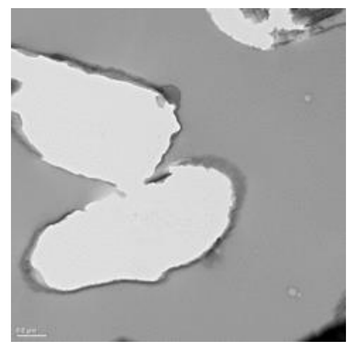

Figure 3
Examples from the FIBSEM stack. HFW = 8 µm.
For obvious reasons the whole 3D volume cannot be displayed at this poster.

Figure 4
Example of cells that were ripped out of the section during sectioning. Example from protocol 5.

Results

The protocol with 1% TCH (protocol 5) had a very high contrast compared to the other protocols. Unfortunately the cells also became very hard to a point where the cells were ripped out of the sectios. This could pose large problems for future 3View work. This was also true to a lesser extent with 0.5% TCH (protocol 4). Protocol 2 and 4 gave similar contrast which points to protocol 2 as a promosing strategy.
The high contrast protocols leave highly electron dense material in the cells and this can hinder the detection of silver nanoparticles. Hence the next step is to use low contrast protocols for comparison and to image freeze-dried CEMOVIS sections.

References
[1] NB Hartmann et al, Aquatic toxicology 118-119 (2012) p. 1.
[2] SN Sørensen and A Baun. Submitted to Nanotoxicology (2014).

Acknowledgement
The authors gratefully acknowledge funding from The Society of Electron Microscope Technology.
Images were acquired at Centre For Ultra Structural Imaging, King's College London and Center for Electron Nanoscopy, Technical University of Denmark.